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Molecular Inclusion Complexation of Tolbutamide with Permethyl-β-cyclodextrin

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Abstract. The formation of a stable inclusion complex between tolbutamide and permethyl-β cyclodextrin was systematically studied. It shows that permethyl-β-CD forms a 1 : 1 complex with tolbutamide. Its molecular structure was elucidated by physicochemical methods including IR and high field NMR analysis. The influence of permethyl-β-cyclodextrin on the hypoglycemic effect of tolbutamide was evaluated by measuring the blood glucose level. The reduction in plasma glucose was significantly greater when the rabbits were treated with the complex than with tolbutamide alone. The enhancement of the bioavailability of tolbutamide by permethyl-β-cyclodextrin is likely attributed to the molecular inclusion effect.

Key words: Inclusion complex, tolbutamide, permethyl-β-cyclodextrin, hypoglycemic effect, glucose level, NMR, FT-IR.

1. Introduction

Cyclodextrins are known to form inclusion complexes with a variety of guest molecules in solution and in the solid state [1]. The minimal requirements for the formation of an inclusion complex are size compatibility and hydrophobicity of the guest molecule. Cyclodextrin complexation has been extensively applied to the enhancement of dissolution rate and bioavailability of poorly soluble drugs [2, 3].

Tolbutamide [1-butyl-3-(*p*-tolylsulfonyl)urea] is a first-generation hypoglycemic drug used clinically in the treatment for insulin-dependent diabetic patients in whom the pancreas retains the capacity to secrete insulin [4]. Its poor water solubility and dissolution rate are considered to be the rate-limiting steps in gastrointestinal absorption. The low water solubility and dissolution rate are likely due to the strong intermolecular hydrogen-bonding between the sulfonylurea groups, which are encircled by two hydrophobic end groups [5]. Modification of tolbutamide dissolution properties by water-soluble polymers has been used to improve oral bioavailability [6–9]. Previous studies suggested that tolbutamide forms 2 : 1 [10, 11] or 1 : 1 [12, 13] inclusion complexes with β-cyclodextrin (β-CD). Cyclodextrin complexation could significantly enhance the water solubility of tolbutamide [14],

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thereby improving the bioavailability of tolbutamide by β-CD as demonstrated by us [10] and others [15, 16].

In 2,3,6-heneicosa-*O*-methyl-β-cyclodextrin, also called permethyl-β-cyclodextrin (permethyl-β-CD), all the hydroxyl groups of cyclodextrin are substituted by methyl groups. Permethyl-β-CD was a more effective solubilizer for poorly water-soluble drugs than the parent β-CD [17]. Ou *et al.* have recently shown that 2,3,6-partially methylated β-CD (methylation ratio: 58–62% at the 2-position, 48–52% at the 3-position and 98–100% at the 6-position) can improve the water solubility of tolbutamide three-fold [18]. Neither the molecular nature of this phenomenon nor its biological effect were described since the methylated β-CD is a complex mixture. We therefore prepared completely methylated β-CD and studied the formation and structure elucidation of permethyl-β-CD–tolbutamide inclusion complex. Its hypoglycemic effect in rabbits was also evaluated.

2. Experimental

2.1. Materials

The following materials were used: β-CD (Chemical Dynamics Corp.), tolbutamide (Sigma Chem. Co.), D_2O (Aldrich Chem. Co.). Their purities were examined by ^{1}H-NMR and used without further purification.

2.2. Spectral data

^{1}H-NMR spectra were recorded on a Nicolet NT-470 MHz spectrometer with 7.7 μs (75°) pulse width and 10 s repetition time. Chemical shifts are reported in part per million relative to 3-(trimethylsilyl)-1-propanesulfonic acid, sodium salt (DSS) (0.0 ppm). All the spin simulations were done on an IBM personal computer using the Raccoon program. Samples for the IR spectral analysis were prepared by using the KBr disc method. IR spectra were measured on a Perkin-Elmer Model 1600 FT-IR spectrophotometer. FAB-MS data were obtained with a Kratos MS-50 sector mass spectrometer utilizing DTT/DTE (3 : 1 dithiothreitol/dithioerythritol) as the ionization matrix.

2.3. Glucose analysis

A Beckman glucose analyzer was used for the quantitative determination of glucose in serum. This quantitation is achieved by measuring oxygen concentration using a Beckman oxygen electrode for this purpose. The method is based upon the following reaction:

$$\text{D-glucose} + O_2 \xrightarrow[H_2O]{\text{glucose oxidase}} \text{glucuronic acid} + H_2O_2$$

An enzymatic reaction [19] of D-glucose with oxygen takes place and the observed rate of oxygen depletion is measured which is proportional to the concentration of

glucose in the sample. Whole blood cannot be used in this assay because it contains viable blood cells that exchange oxygen with enzyme reagent and thus interferes with the oxygen rate method; therefore, the whole blood was centrifuged before being analyzed and the serum was used for blood glucose determination.

2.4. SYNTHESIS OF PERMETHYL-β-CD (2,3,6-HENEICOSA-*O*-METHYL-β-CYCLODEXTRIN)

Following the modified procedure of Szejtli *et al.* [20], a mixture of 5 g (4.4 mmol) of anhydrous β-CD, 7 g of sodium hydride and 150 mL of anhydrous dimethylformamide was stirred under nitrogen at room temperature for 3 h. The reaction mixture was cooled to 0°C, and 40 mL of methyl iodide was added dropwise. This solution was stirred under an atmosphere of nitrogen for another 6 h at room temperature. The mixture was filtered and evaporated to dryness under high vacuum below 50°C. The solid residue was dissolved in 50 mL of ice water and extracted 5 times with 200 mL of chloroform. The combined chloroform solution was evaporated to dryness under reduced pressure at 30°C. The resulting crude product was further purified on a silica gel column and eluted with toluene/l-propanol (5 : 3) to yield the permethyl-β-CD–toluene complex. The toluene was then removed under high vacuum at 110°C to obtain 3.5 g (55.5%) of solid product, which was further crystallized from cyclohexane, and dried under high vacuum at 90°C. Mp: 153–154°C (152–154°C [20]). FAB-MS (3 : 1 DTT/DTE) m/z 1451.5, 1397.5, 1366.5. IR and ^{1}H-NMR spectral analyses are shown in Figures 1(b) and 2.

2.5. PREPARATION OF PERMETHYL-β-CD–TOLBUTAMIDE INCLUSION COMPLEX

Equal mmolar amounts of permethyl-β-CD (1.43 g) and tolbutamide (0.27 g) were dissolved in 50 mL of pH 11.0, 0.05 M phosphate buffer. This solution was stirred at room temperature for 30 mm and then neutralized with 1.0 N HCl. Following neutralization the solution was stirred at room temperature for several hours and concentrated under reduced pressure until cloudiness appeared. This solution was then cooled to room temperature and filtered. The filtrate was placed in a water bath at 50°C for several days. The resulting crystals were shown to be the permethyl-β-CD–tolbutamide (1 : 1) complex by ^{1}H-NMR. Further recrystallization from water at 50°C also formed a 1 : 1 complex. Mp: 127–128°C. FT-IR and ^{1}H-NMR spectral analyses are shown in Figures 1(d) and 3.

2.6. BIOAVAILABILITY STUDY

The modulation of the bioavailability of tolbutamide by permethyl-β-CD was monitored by measuring its hypoglycemic effect in male New Zealand white rabbits. Seven rabbits were used per group. One group of rabbits was treated with tolbutamide (50.0 mg/kg = 0.185 meq/kg) only, the other group of rabbits was treated

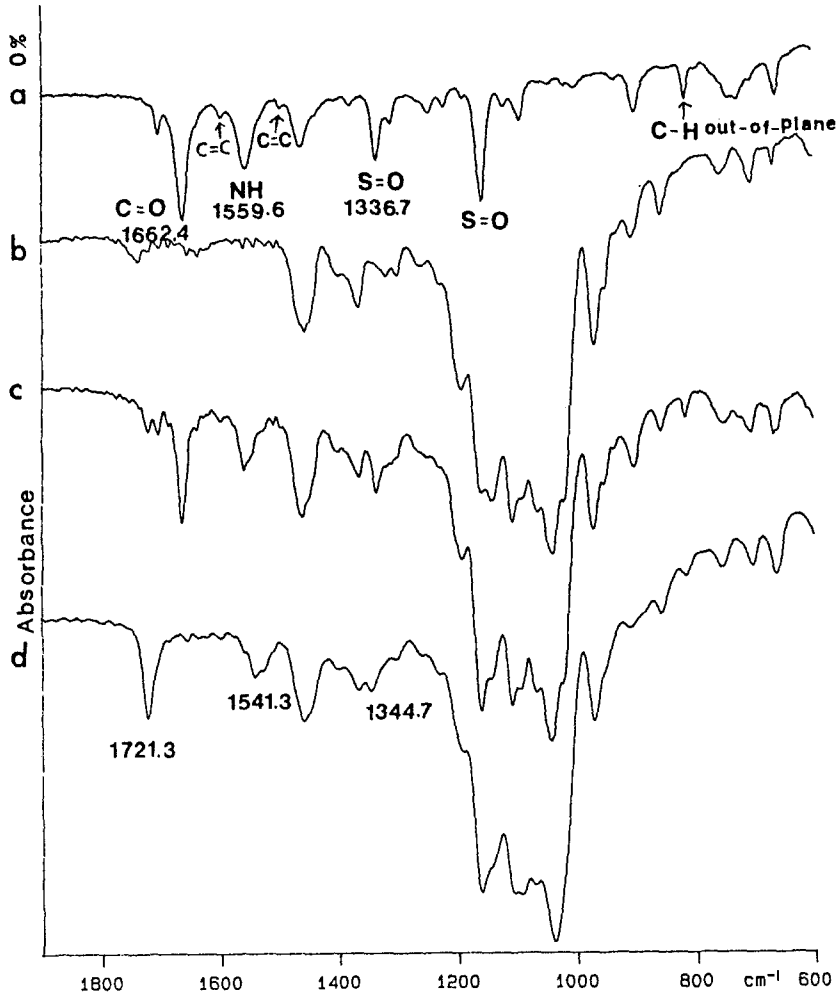


Figure 1. IR absorption spectra of: (a) tolbutamide; (b) permethyl-β-CD; (c) permethyl-β-CD + tolbutamide (1 : 1) physical mixture; (d) permethyl-β-CD–tolbutamide (1 : 1) complex.

with permethyl-β-CD–tolbutamide (317.8 mg/kg = 0.187 meq/kg). Before administering the drug, rabbits were denied food for 24 h. Blood samples were taken before administering the drug, 30 min after oral administration by intubation, and at 1 h intervals for 8 h. A Beckman glucose analyzer was used to assay the plasma glucose level.

Blood glucose levels were determined from 30 min to 8 h after drug administration. Curves were fitted to fourth-order polynomials. Serum areas under concentration-time curves (AUC) were calculated using the *Mathematica* program (Wolfram Research Inc., Champaign, Illinois). Means of AUC and the concentration of blood

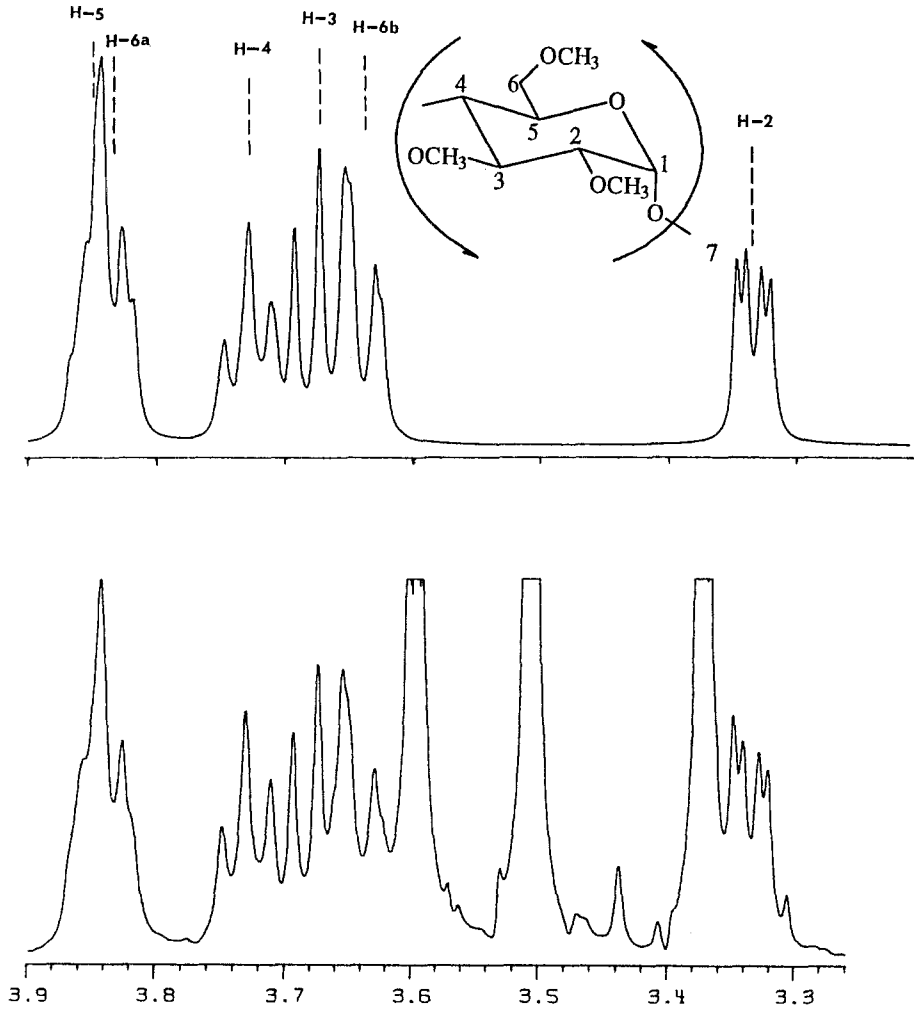


Figure 2. 470 MHz ^{1}H-NMR spectrum of permethyl-β-CD (bottom) and its spin simulated spectrum (upper). The spin simulated spectrum is shown without three methoxy groups.

sugar at each time point of these two treatment groups were compared using Student's two-tailed t-test with a chosen level of significance of $P < 0.05$.

3. Results and Discussion

The FAB-MS spectrum of permethyl-β-CD showed a distinct peak at m/z 1451.5 derived from the sodium adduct of the molecular ion. The peak at m/z 1397.5 was assigned to a fragment from the protonated permethyl-β-CD ion (m/z 1429.5) losing one molecule of methanol or from the permethyl-β-CD cation radical (m/z 1428.5) losing one methoxyl radical. The peak at m/z 1366.5 could be assigned to the degradation fragment from the removal of a methoxyl radical from the

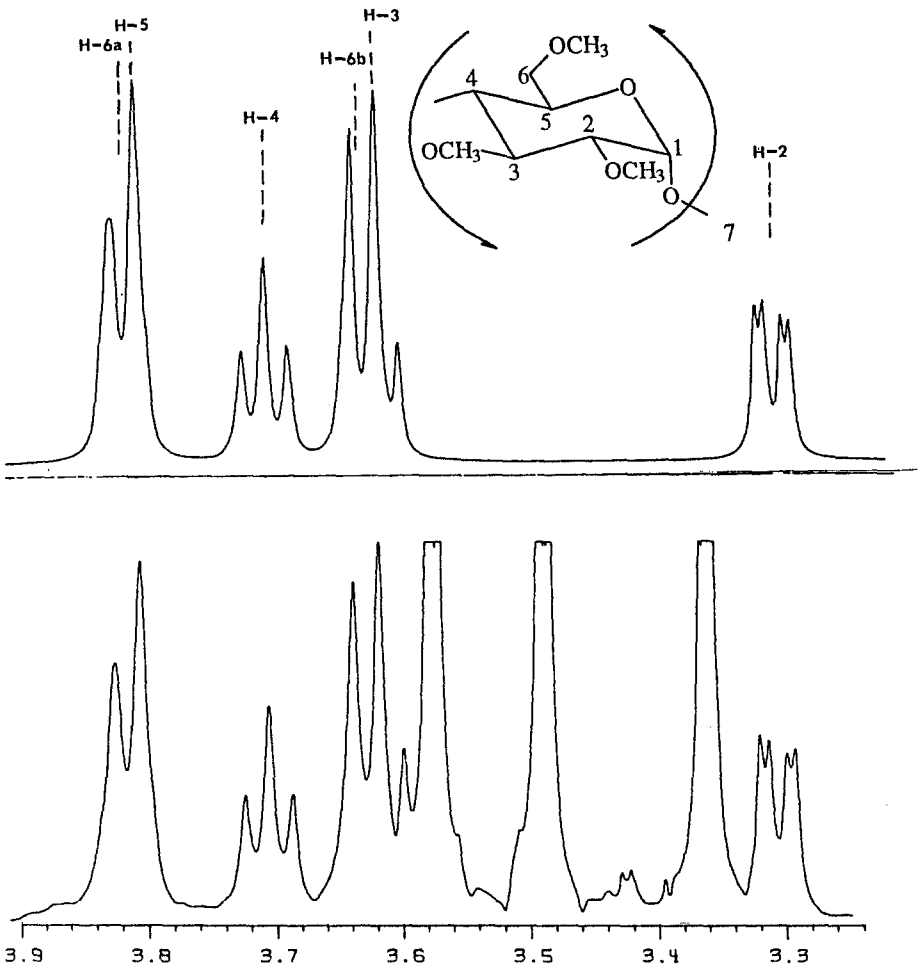


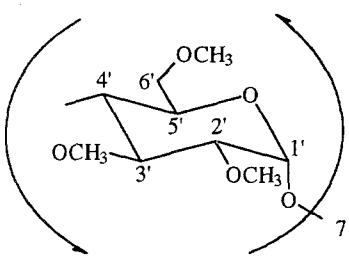
Figure 3. 470 MHz ^{1}H-NMR spectrum of permethyl-β-CD–tolbutamide (bottom) and its spin simulated spectrum (upper). The spin simulated spectrum is shown without three methoxy groups.

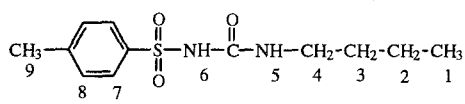
m/z 1397.5 fragment. There were no significant peaks at m/z 1437.5, 1423.5, 1409.5,..., etc. corresponding to the incomplete methylation products. Furthermore, the spectral data and peak intensity of the 470 MHz ^{1}H-NMR spectrum provided a definitive proof that all hydroxyl groups were methylated (Table I). The chemical shift for all methine and methylene protons are unambiguously assigned by the computer aided spin-simulation as shown in Figure 2.

The permethyl-β-CD–tolbutamide inclusion complex was prepared by crystallization from water at 50°C and shown to be a 1 : 1 host-guest complex by

Table I. 470 MHz ^{1}H-NMR chemical shifts (ppm) of tolbutamide, permethyl-β-CD and the permethyl-β-CD–tolbutamide complex (1 : 1) in 0.2 N NaOD solution.

Protons	Permethyl-β-CD–tolbutamide (ppm)	Permethyl-β-CD (ppm)	Tolbutamide (ppm)	Chemical shift difference (Hz)
1′	5.2445	5.2665		−10.3
2′	3.3080	3.3330		−11.7
3′	3.6200	3.6740		−25.3
4′	3.7065	3.7270		−9.6
5′	3.8130	3.8495		−17.1
6′a	3.8190	3.8325		−6.3
6′b	3.6300	3.6385		−4.0
2-OCH_3	3.5740	3.5930		−8.9
3-OCH_3	3.4880	3.5280		−18.8
6-OCH_3	3.3610	3.3680		−3.3
1	0.8270		0.8080	8.9
2	1.2025		1.2050	−1.2
3	1.3370		1.3300	3.3
4	2.9760		2.9620	6.6
7	7.7005		7.6800	9.6
8	7.3285		7.3265	0.9
9	2.3790		2.3600	8.9





^{1}H-NMR analysis. The molar composition of this complex remained constant after recrystallization.

Its FT-IR spectrum was compared with the spectrum of free tolbutamide, permethyl-β-CD, and a physical mixture of these two compounds (Figure 1). The spectrum of the physical mixture shows a carbonyl stretching absorption at 1663.3 cm^{-1} and an NH bending absorption at 1559.8 cm^{-1}, which are almost identical to the absorptions of free tolbutamide (1662.4 cm^{-1} and 1559.6 cm^{-1}). X-ray studies [21] show that tolbutamide possesses intermolecular hydrogen bondings between the C=O group of one molecule and the N—H group of another molecule, also between the S=O group of one molecule and the N—H group of another molecule. The spectrum of the inclusion complex displays the carbonyl stretching absorption shifted to a higher wave number, 1721.3 cm^{-1} ($\Delta\nu$: 58.9

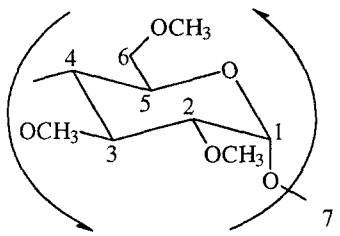
cm^{-1}); and the NH bending absorption is shifted to a lower wave number, 1541.3 cm^{-1} ($\Delta\nu$: -18.3 cm^{-1}) A similar shift for the C=O absorption was also observed in the complexation of tolbutamide with β-CD [10]. These IR absorption shifts are likely attributable to the disruption of the strong intermolecular hydrogen bondings between the sulfonylureido functional groups in free tolbutamide by the formation of the cyclodextrin complex. These distinct shifts suggest that tolbutamide is incorporated as a molecular complex not a physical mixture. The absorption intensity of the complex is also significantly reduced, probably due to the vibrational restriction of tolbutamide in the cyclodextrin complex. These unique IR changes, however, cannot definitely indicate the formation of an inclusion complex.

The 470-MHz ^{1}H-NMR spectra of permethyl-β-CD and permethyl-β-CD–tolbutamide complex in 0.2 N NaOD solution shows highly second-order spectral characteristics for the cylcodextrin proton resonances which results in severe signal overlap (Figures 2 and 3). Direct spin simulations were thus employed to determine the precise coupling constants and chemical shifts. Since the RACCOON spin simulation program can only simulate a spin system containing seven spins, we could thus not include the methyl protons in the spin simulation of the ring protons of glucose. In any case, the four-bond coupling effect between the methyl protons and their neighboring glucose protons is very small and should not significantly affect the simulated spectrum. The experimental and simulated spectra of both permethyl-β-CD and permethyl-β-CD–tolbutamide are shown in Figures 2 and 3, respectively. Upon complexation with permethyl-β-CD, marked spectral changes of tolbutamide were observed (Table I). The downfield shifts of all the phenyl protons may be ascribed to the absence of aromatic ring stacking and the variation of dielectric environment upon complexation, which are consistent with previous studies of cyclodextrin complexation with other aromatic molecules [22]. In contrast to the downfield shift, β-CD induced a strong upfield shift for the *meta*-protons of the tolbutamide phenyl ring. This upfield shift may be ascribed to the shielding effect of the ether oxygen (O-4′) of β-CD [10].

The upfield shift for the H-3′ and H-5′ of permethyl-β-CD upon the formation of tolbutamide inclusion complex (Table I) can be attributed to the anisotropic shielding effect of the phenyl ring. This anisotropic shielding offers a valuable approach to determine the spatial disposition of the aromatic group in the complex by comparing the relative magnitude of the induced chemical shift changes for H-3′ and H-5′ [23, 24]. The larger shift of H-3′ (25.3 Hz), relative to the shift of H-5′ (17.1 Hz), suggests that the aromatic ring is situated closer to the H-3′ plane than the H-5′ plane. This is different from our previous study of the β-CD–tolbutamide inclusion complex [10]. The steric hindrance of the 2′,3′-dimethoxy groups of permethyl-β-CD is likely to play a significant role in the complexation with tolbutamide. In addition, H-1′, H-2′ and H-4′ of the permethyl-β-CD–tolbutamide complex are slightly shifted upfield, whereas the corresponding signals of the β-CD–tolbutamide complex remain constant [10]. This implies that a small portion of the aromatic rings may also stay outside of the cavity. Moreover, the

Table II. 470 MHz ^{1}H-NMR coupling constants (Hz) of permethyl-β-CD and permethyl-β-CD–tolbutamide complex in 0.2 N NaOD solution.

Coupling constants	Permethyl-β-CD (Hz)	Permethyl-β-CD–tolbutamide (1 : 1) (Hz)
J_{12}	3.5	3.1
J_{15}	−0.5	1.5
J_{23}	9.4	9.5
J_{34}	9.0	8.5
J_{45}	9.0	9.5
J_{46a}	−1.5	−1.0
J_{46b}	−0.5	0.0
J_{56a}	4.0	3.0
J_{56b}	2.5	2.5
J_{6a6b}	−11.3	−10.3



downfield shifts for both aromatic protons and aliphatic protons of tolbutamide suggest that both groups may be included in the cavity. Therefore two plausible dispositions of tolbutamide in the permethyl-β-CD–tolbutamide complex may be envisioned, as shown in Figure 4. Alternatively, permethyl-β-CD may complex with tolbutamide in a channel form or two moles of permethyl-β-CD may form a 2 : 1 complex with one mole of tolbutamide (Figure 5). However, we were thus far unable to obtain a stable 2 : 1 complex in the solid state.

The vicinal coupling constants (J_{12} to J_{45}) for the modified glucose units of permethyl-β-CD are not significantly changed upon complexation with tolbutamide (Table II), suggesting that the inclusion of tolbutamide into the permethyl-β-CD cavity does not lead to significant distortion of the C1-chair conformation of glucose. Similar results were also found in the β-CD–tolbutamide [10], β-CD–benzaldehyde [25], α-CD–benzaldehyde [10], α-CD–penicillin V [26] and α-CD–*p*-iodoaniline complexes [27]. X-ray crystallographic studies [28] also suggest that the glucose units in cylcodextrin behave as relative rigid building blocks, with the main conformational freedom being rotation about the C1—O4, C4—O4 and C5—C6 bonds [29].

The influence of permethyl-β-CD on the hypoglycemic effect of tolbutamide was studied by monitoring blood glucose levels in rabbits. The blood glucose

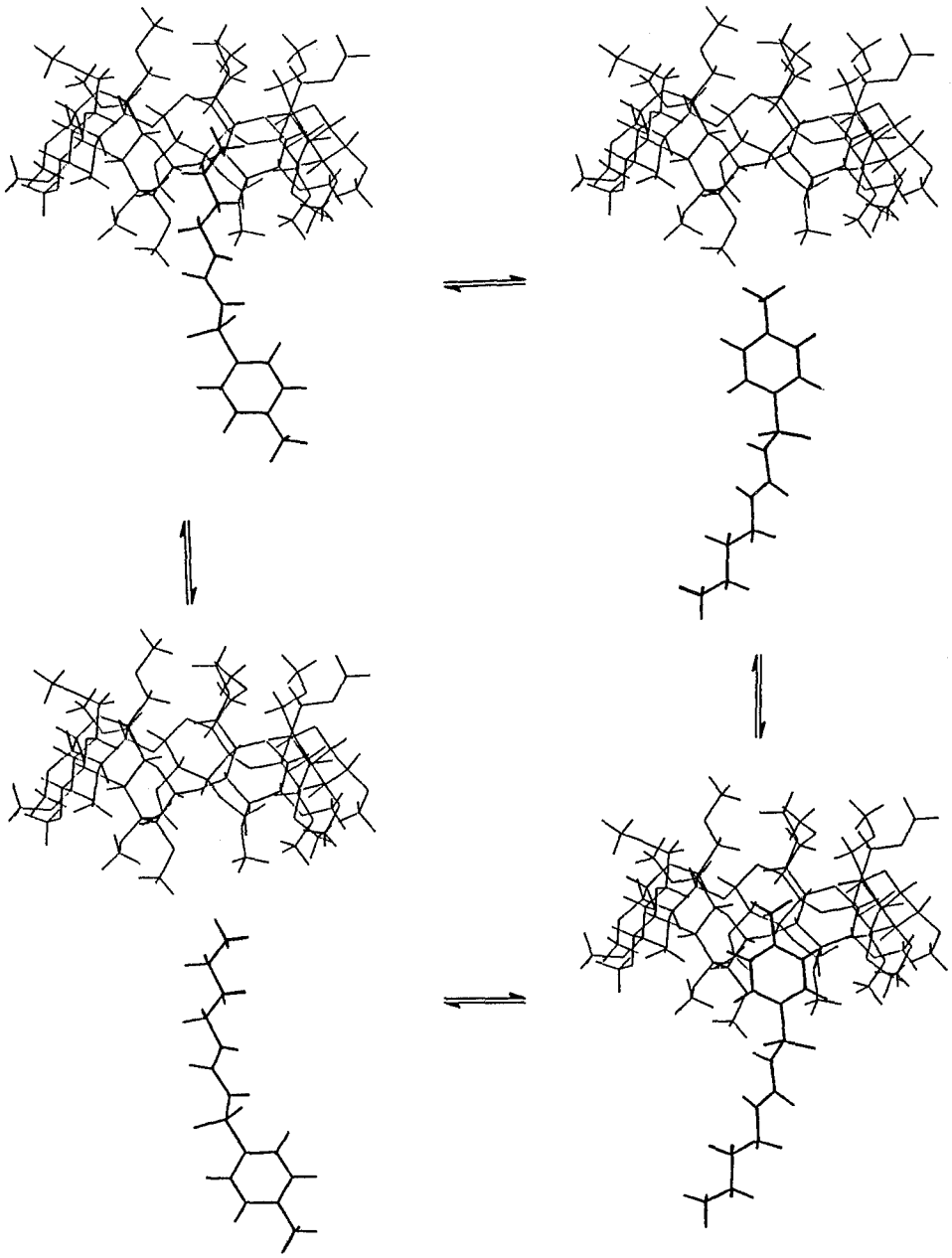


Figure 4. Potential intermolecular interactions for the permethyl-β-CD–tolbutamide complex.

levels versus time after administration of the drugs are shown in Figure 6. After 1 h, blood glucose levels in the rabbits treated with permethyl-β-CD–tolbutamide were significantly lower than those in rabbits treated with tolbutamide alone. When

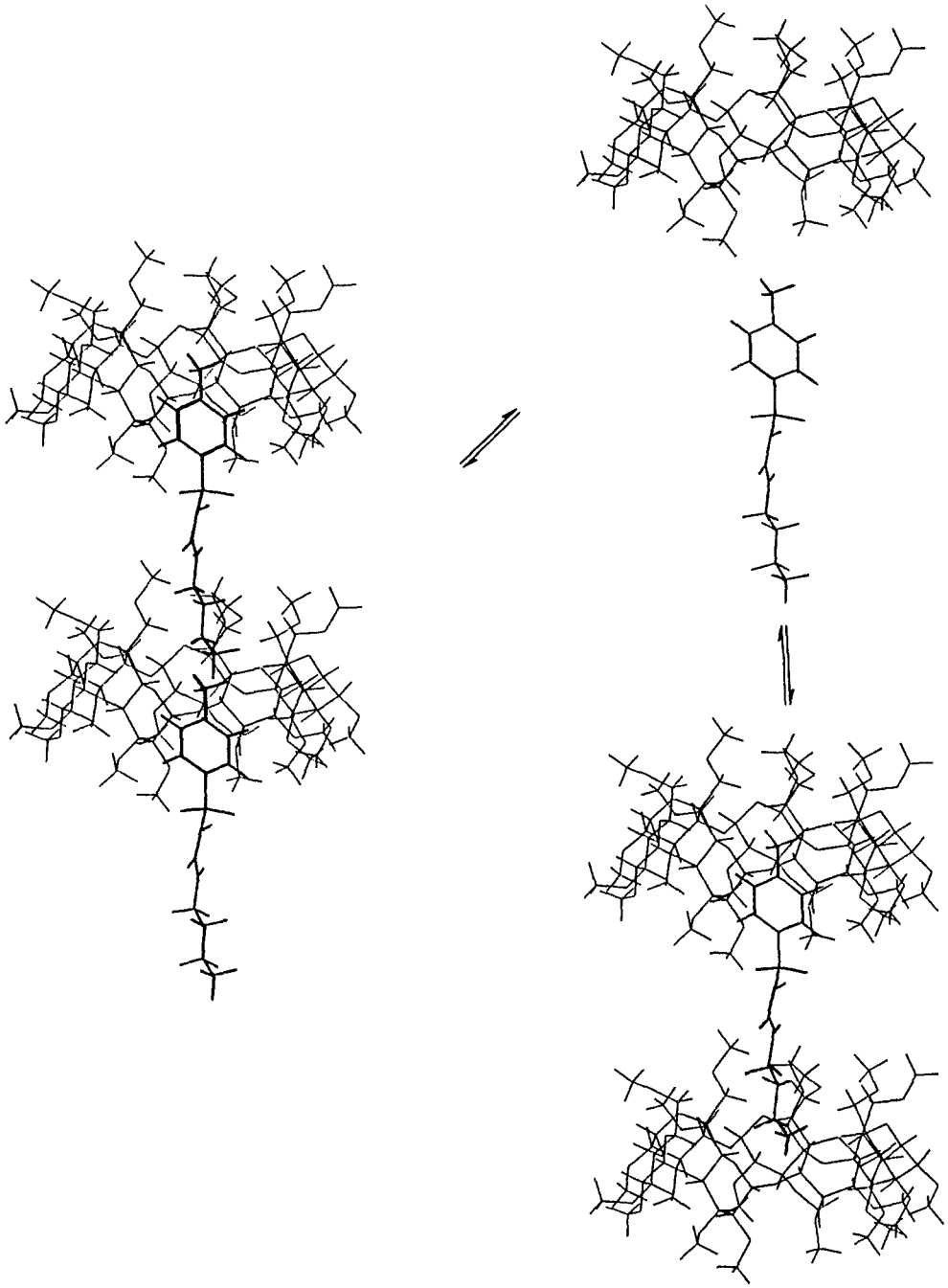


Figure 5. Alternative structural disposition for the permethyl-β-CD–tolbutamide complex.

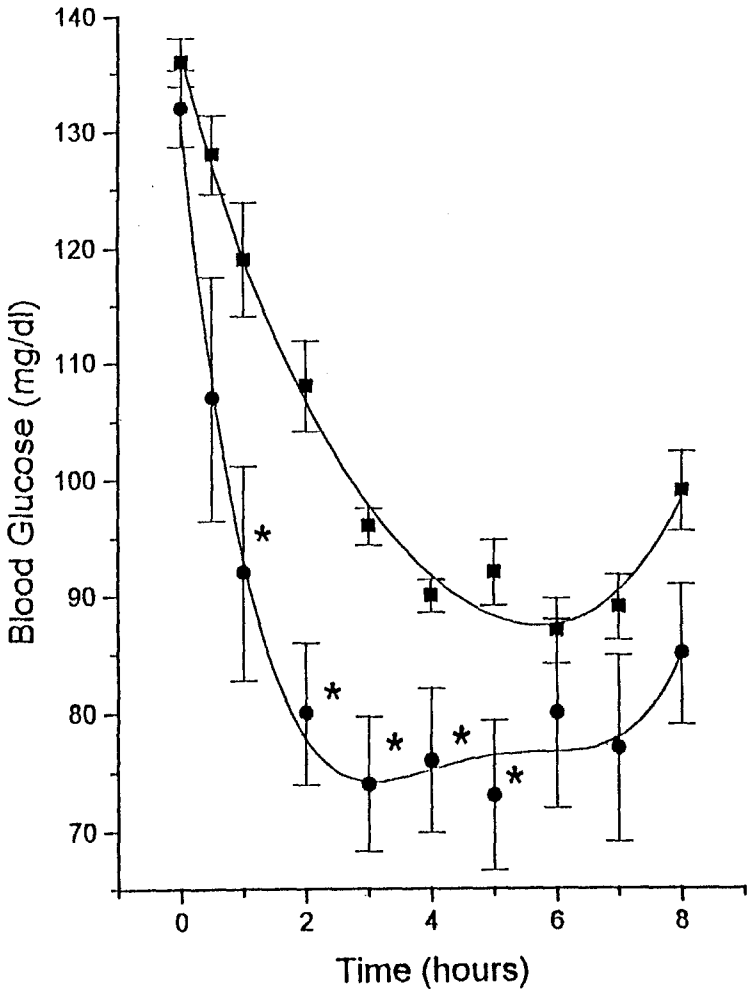


Figure 6. Blood glucose levels versus time after administration of tolbutamide (■) and permethyl-β-CD–tolbutamide (●). Values are mean ±S.E. for groups of 7 rabbits. An asterisk indicates a significant difference between treatment groups ($P < 0.05$). Curves are fitted with fourth-order polynomials with an R value of 0.99.

tolbutamide was given alone, the lowest plasma glucose level was obtained after 6 h (87 ± 2.8 mg/dL), while tolbutamide administered in the permethyl-β-CD inclusion complex resulted in a nearly maximal hypoglycemic effect in 3 h (74 ± 5.7 mg/dL). According to the areas under serum concentration time curves (AUC), it is clear that the decrease in plasma glucose was significantly greater ($p < 0.05$) when the rabbits were treated with permethyl-β-CD complex (AUC = 654 ± 44 mg/dL) than when given the tolbutamide alone (AUC = 796 ± 16 mg/dL). The better hypoglycemic effect of permethyl-β-CD–tolbutamide than the free-form of

tolbutamide might be attributed to the solubilizing effect of permethyl-β-CD on tolbutamide [18].

4. Conclusions

In summary, tolbutamide can form a stable inclusion complex with permethyl-β-CD in solution and in the solid state. This molecular complexation enhances the solubility of tolbutamide and may therefore contribute to the improvement of the bioavailability of tolbutamide.

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